SOME EFFECTS OF ETHYLENE ON THE METABOLISM OF PLANTS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 207

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(WITH TWO FIGURES)

Introduction

Etiolated pea seedlings develop abnormally when they are grown in the "impure air" of a laboratory. This response to atmospheric impurities has become well known through the work of NELJUBOW (25) and others. Three phases, at least, are usually distinguished in the response of the epicotyl of the seedling: (1) a retardation in the rate of elongation, (2) swelling, and (3) a change from negative geotropism to diageotropism. Furthermore, this characteristic response can be produced by a large number of chemical compounds. However, the three phases mentioned are not induced with equal ease; the third may never appear for a given substance, although that substance readily causes swelling and interferes with the rate of elongation. Likewise, both the second and the third may not appear, although there is a marked retardation of growth. The swelling of developing plant organs in the presence of poisonous substances is a very common response, especially when the concentration of the substance in question is near the lower toxic limit. So frequently does this phenomenon occur that one is perhaps justified in saying that swelling is one of the first superficial indices of a disturbance in the metabolism of a plant.

Of the large number of chemical compounds capable of inducing swelling in the pea seedling, ethylene has been found to be the most effective. According to KNIGHT, ROSE, and CROCKER (19), ethylene will cause swelling of the epicotyl of the sweet pea seedling in dilutions of about 0.00004 per cent (by volume), while to produce similar results with chloroform, for example, the concentration

must be about 1 per cent. This illustrates how sensitive the sweet pea seedling is to traces of ethylene. Moreover, it seems fairly well demonstrated that for plants in general (but by no means all) ethylene is relatively very toxic.

The remarkable capacity of ethylene to induce swelling naturally suggests the question, What is the effect of ethylene on plant metabolism? A certain amount of work has been published on the effects of illuminating gas and "laboratory air," which should furnish required data on the question, since in both those gaseous mixtures ethylene has probably been an important factor. Nevertheless, with regard to the effect of ethylene as such, I have been able to find no literature. This was largely the reason for undertaking the investigation reported here, the work having as its subject the determination of the changes brought about in plant tissue by ethylene.

Historical

Our knowledge of the changes in metabolism causing and accompanying swelling of plant organs has been gained largely from investigation of the effects of anaesthetics, particularly ether and chloroform. For a general historical résumé of the literature of the effects of anaesthetics on plants, the reader is referred to an excellent paper by HEMPEL (11). The following consideration of the literature deals only with the effects of anaesthetics on the chemical composition and the respiratory processes.

JOHANNSEN (16, 17) found that certain concentrations of ether and chloroform caused an increase of soluble sugars and a decomposition of proteins in bulbs of *Crocus* and seeds of pea and barley. But he also noted that very weak ether gave reversed effects, that is to say, favored starch and protein synthesis. His explanation for the increase in sugars and amino bodies was simply that anaesthetics interfered with the condensation, but not with the hydrolyzing processes. ZALESKI (36), working with *Lupinus*, found that protein synthesis was favored by ether and hindered by caffein. BUTKEWITSCH (3) and BARTEL (2) both reported an increase of tyrosin in *Lupinus*, as an effect of chloroform. PRIANISCHNIKOW (28) was able to demonstrate a considerable increase of asparagin in *Lupinus* when the seedlings were grown in an atmosphere containing

traces of gaseous impurities. LESCHTSCH (**22**) studied the effect of turpentine on protein metabolism in bulbs of *Allium*. An acceleration of protein synthesis occurs in wounded bulbs, but the process is further accelerated by small amounts of turpentine and hindered by large amounts. PURIEWITSCH (**27**) noted that ether interfered with the synthesis of starch. To account for this phenomenon he assumed an increased rate of respiration, whereby the sugars were used up. BUTKEWITSCH (**4**) reports similar effects for toluol and chloroform. Starch in the bark and wood of *Morus* and *Sophora* was rapidly hydrolyzed. This hydrolysis cannot be explained, he thinks, on the supposition of an increased respiration, since sugars increase concomitantly with the decrease of starch. Also, he points out the analogy between the effects of toluol and chloroform and of low temperatures; both may be explained on the basis of injury to the plastids. REINHARD and SUSCHKOFF (**29**) determined the effects of several substances upon starch synthesis. Ether seemed to act, not only as a hindrance to starch formation, but also as an accelerator of hydrolysis. Antipyrin, morphine, and caffein hindered, but urea and asparagin favored starch synthesis. Similarly DELEANO (**6**) observed a rapid destarching of leaves in the presence of chloroform, a result apparently contradictory to that reported by CZAPEK (**5**). RICHTER (**30**), working with illuminating gas, laboratory air, xylol, etc., and GRAFE (**8**), with formaldehyde, have shown that an accumulation of sugar is favored by these substances. ARMSTRONG and ARMSTRONG (**1**) have demonstrated that toluol, ether, chloroform, etc., cause an increase of glucose and HCN in leaves of *Prunus lauro-cerasus*, due to a rapid splitting of the glucoside present. HEMPEL (**11**) has made a careful study of the effects of ether on seedlings of *Pisum* and *Lupinus* with particular regard to the CO_2 output, and the changes in the nitrogen compounds and sugars. Her results show that ether effects are dependent upon the concentration. The normal destruction of the proteins in germination was retarded by "weak" doses (up to approximately 0.01 per cent by volume), but the process was accelerated in strong doses. All concentrations interfered with the inversion of sugars. GRAFE and RICHTER (**9**) have published an article on the effects of acetylene on the chemical

composition of several kinds of seeds and shoots. They found that the sugars and amino acids increased in tissues which were naturally high in carbohydrate (*Vicia*, *Laburnum*, and potato shoots), while in fatty tissue (seeds of squash, mustard, and flax) there was a slight decrease. Also, acetylene caused an increase in the amount of glycerine and fatty acid in the seeds, resulting in a decrease in the amount of fat. Like results were obtained for illuminating gas. They conclude that the condensation processes alone are affected. However, it seems quite possible that anaesthetics sometimes also hasten the hydrolyzing processes. This is further indicated by the recent work of McCool (**24**), in which he claims that the acceleration of enzymatic activity (of diastase and oxidase) takes place during etherization, although the activity of catalase is depressed.

A preliminary examination of the results referred to above shows a number of inconsistencies; but Grafe and Richter have well pointed out that these inconsistencies are probably not real. Most of them become clear when the effect of anaesthetics, with regard to the general chemical reactions of plants, is expressed as follows: that the condensation processes are favored by "weak" and hindered by "strong" concentrations; but that the effect on the hydrolyzing process is uncertain.

The literature dealing with the effects of anaesthetics on respiration processes uncovers about the same general situation as stated above, since weak doses seem to accelerate and strong doses to retard respiration. This statement is borne out by the results of Elfving (**7**), Johannsen (**15**), Morkowin (**23**), Laurén (**21**), and others. However, Morkowin considers that the respiration of carbohydrates cannot be accelerated by ether; that such is possible only with nitrogenous substances. Also Laurén found that whether or not respiration could be accelerated by ether depended upon the kind of plant used. Respiration was accelerated in proportion to the dose in *Ricinus* and *Lupinus;* slightly accelerated in limited doses, later depressive, in *Pisum*, *Phaseolus*, and *Cucurbita;* and there was no acceleration in *Brassica*, *Hordeum*, and *Zea*. Irving (**12**) has shown that for chloroform the effect depended upon the dose. Small doses increased the CO_2 releasal; medium doses

caused an initial outburst, afterward a falling off; and strong doses caused depression from the beginning. THODAY (33) has made some careful determinations, both of CO_2 releasal and of O_2 absorption. Weak concentration of chloroform accelerated both processes to an equal degree, hence the respiration ratio remained the same. When the doses were considerably stronger, respiration was retarded, but the correlation between the two processes was broken up. In leaves without tannin (*Tropaeolum*) O_2 absorption was depressed more than the CO_2 output. But in leaves containing tannin (*Pinus* and *Helianthus*) the situation was reversed; there was an initial rapid absorption of O_2 which soon fell to a level somewhat above the CO_2 production.

Material

The sweet pea seedling was chosen as experimental material for the present study, largely because it is so sensitive to toxic substances, and on account of the general interest surrounding its characteristic responses.

Etiolated seedlings were used throughout the experiments. The seeds were purchased under the trade name Gladys Unwin (Vaughn's Seed Store). The cultural methods employed have been described by KNIGHT and CROCKER (20), although some minor changes were necessary in order to care for large cultures. The methods are briefly outlined below.

The seeds were scratched with a file (to secure quick and uniform germination), soaked for 12 hours in distilled water, and germinated on wet filter paper. When the hypocotyls had become 3–7 cm. long the seeds were sowed upon wet absorbent cotton in large pans (2×30×48 cm.) and covered with a layer of wet filter paper. They were allowed to develop in absolute darkness at a temperature of 21–24° C., until the epicotyls had reached an average length of about 2 cm. The filter paper was then taken off the seedlings and the culture equally divided into two portions, one for treatment with ethylene, the other for control. The entire culture usually consisted of 12 pans, each containing about 250 seedlings. The portion for ethylene treatment was transferred to a galvanized iron box of 225 liters' capacity; the lid sealed gas-

tight; and enough ethylene[1] admitted to make the concentration about 0.0001 per cent by volume. Both control and treated portions were then allowed to continue development for 72 hours under the same conditions (that is, of moisture, temperature, and darkness), except for the ethylene in one. At the end of this period the epicotyls were collected for experimentation. The total culture period was 9 days.

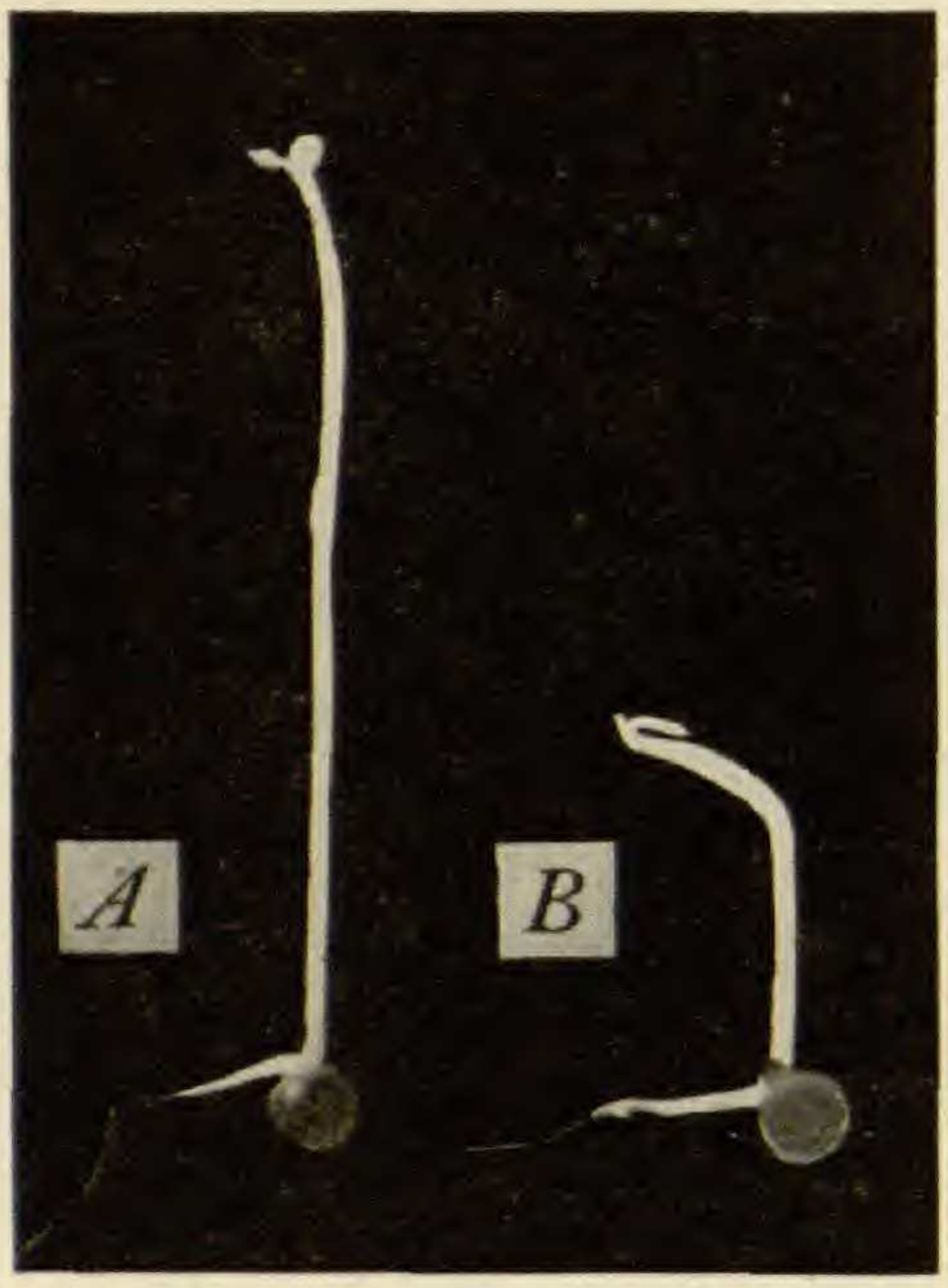

FIG. 1.—Etiolated seedlings of the sweet pea, showing the stage of development at which the epicotyls were taken for experimentation: *A*, normal; *B*, ethylene treated, showing the "triple response"; $\times\frac{1}{2}$.

The epicotyls of the control seedlings, at the time of collection, were 8–11 cm. in length and vertical and straight. But the ethylene treated seedlings showed the well known "horizontal nutation" (NELJUBOW **25**) or "triple response" (KNIGHT and CROCKER); that is, the epicotyls were only 3–5 cm. long, swollen, and had assumed horizontal or nearly horizontal positions. The difference in appearance between the ethylene treated and the untreated seedlings is shown in fig. 1. Certain histological differences are shown by the drawings of fig. 2.

Methods and experimentation

The present attack of the problem on the effects of ethylene has been made through a study of the following questions: (1) chemical composition, (2) acidity, (3) osmotic pressure and permeability, and (4) respiration. A decided emphasis has been laid upon the chemical phase of the problem.

[1] The ethylene used in these experiments was prepared by dropping ethyl alcohol into syrupy phosphoric acid at a temperature of about 215° C. The final dilutions of ethylene were made from a stock ethylene-air mixture containing 2.5 per cent ethylene.

A. CHEMICAL ANALYSIS

1. *General procedure*

At the close of the culture period, the epicotyls of the seedlings were collected, their wet weight determined, and preserved in

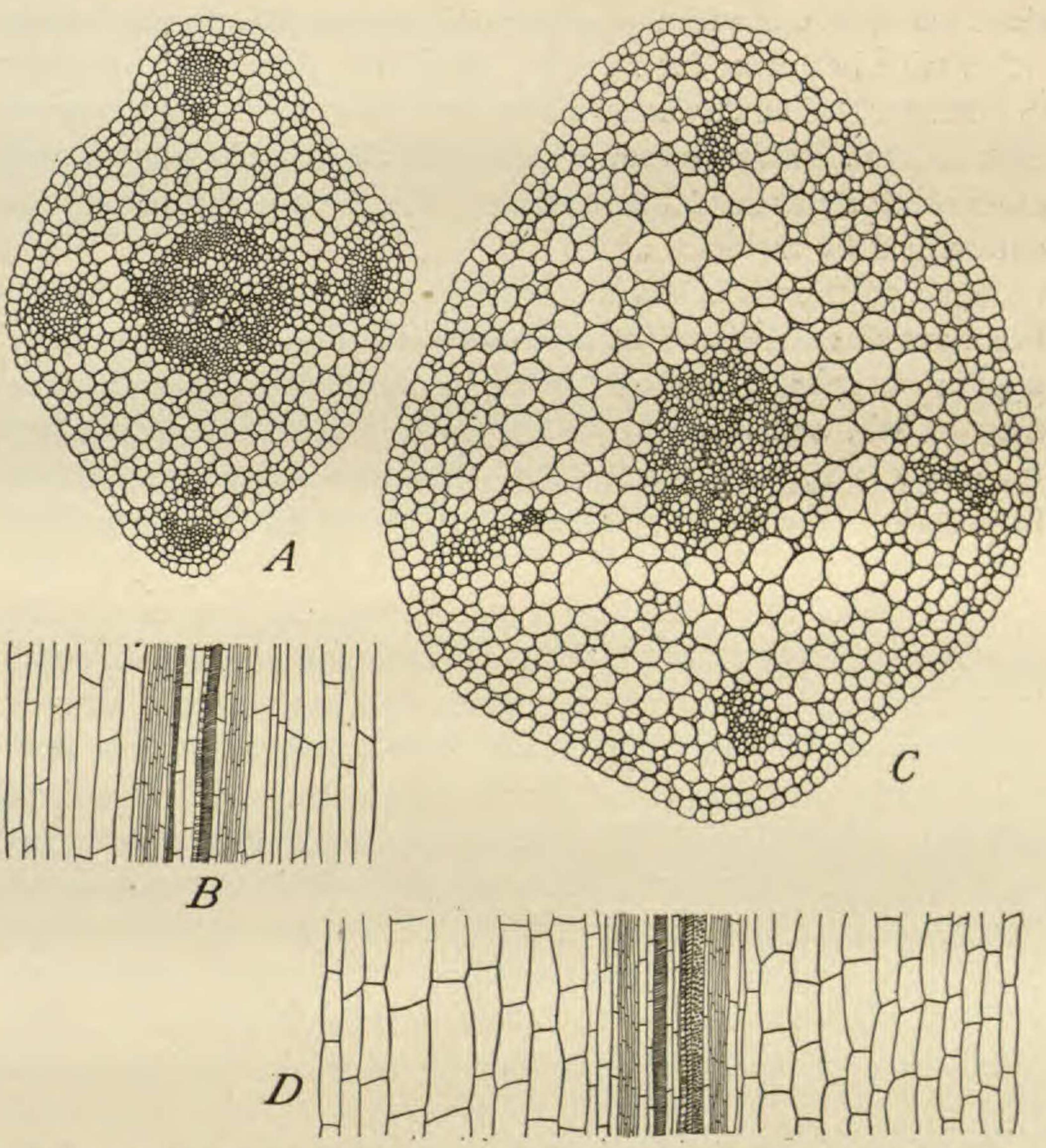

FIG. 2.—Sections of the sweet pea epicotyl at the stage corresponding to fig. 1: *A* and *B*, normal; *C* and *D*, ethylene treated; ×50.

85 per cent alcohol[2] (redistilled). Both the ethylene treated and the untreated samples usually weighed 100–150 gm. Soon after

[2] Enough 95 per cent alcohol was added to make the final concentration 85 per cent, allowing for water in the tissue, which was about 92 per cent.

preserving, the tissue was heated in a water bath for one hour at 70° C. and set aside for at least one week before proceeding with the analysis. Later the epicotyls were cut with scissors into 2–5 mm. lengths, transferred to ashless filter paper extraction cups, and the preserving liquid filtered through the cups. Two extractions followed, one with hot 95 per cent alcohol for 4 hours and the other with hot ether for 2 hours. Then the tissue was powdered in a mortar and finally extracted for 12 hours more with hot 95 per cent alcohol. This procedure separated the sample into alcohol-ether soluble and insoluble portions. The dry weight of each was determined by methods which are described below. That of the insoluble fraction was found simply by drying to constant weight in an oven at 104° C. But the soluble fraction was concentrated upon a water bath to about 450 cc., transferred to a 500 cc. volumetric flask, and made up to the mark. Then an aliquot part (usually 100 cc.) was taken for dry weight determination, and later for ashing. The final drying was carried out in a vacuum desiccator over $CaCl_2$.

Analysis of the alcohol-ether soluble fraction was carried out on the remaining 400 cc. This was evaporated to small volume to free it from alcohol, taken up with water, and transferred to a 500 cc. volumetric flask. Fats and lipoids were precipitated from solution by the addition of 3–5 cc. of chloroform and 10–15 cc. of 5 N HC. After shaking, the solution was made up to volume and set aside in an ice box for 24 hours to settle. The clear supernatant liquid was then decanted and filtered. No determinations were made upon this fat and lipoid residue. The water solution was divided into portions for the following determinations: (*a*) carbohydrates, (*b*) total nitrogen, and (*c*) ammonia and alpha-NH_2 nitrogen.

Analyses on the alcohol-ether insoluble fraction included the following: (*a*) reducing sugars after acid hydrolysis, (*b*) total nitrogen of the fraction, (*c*) total nitrogen of the portion rendered soluble by acid hydrolysis, (*d*) "crude fiber" (dry weight only), which was that portion remaining insoluble after acid hydrolysis, and (*e*) weight of ash. The "crude fiber" would largely be cellulose plus protein which was yet undissolved by acid hydrolysis, hence the results

from $(d)-[(b)-(c)]$ should give an approximate figure for the cellulose present.

2. *Carbohydrates*

Reducing sugars, before and after hydrolysis, were determined for the alcohol-ether soluble fraction. The methods employed were those of MUNSON and WALKER (**26,** pp. 241–251). The reduced copper was determined by the volumetric permanganate method described in the above-named bulletin (p. 52). Similarly, the reducing sugars were determined for the insoluble fraction after 2.5 hours of hydrolysis with 3.5 per cent HCl.

3. *Nitrogenous substances*

Total nitrogen was determined by the KJELDAHL method and the amino acids and amids by both the formol titration (as described by JESSEN-HANSEN, **14,** and VAN SLYKE'S methods, **34**). While employing the latter method, estimations were made of ammonia and alpha-NH_2 groups both before and after a 24-hour hydrolysis with 20 per cent HCl at 98–99° C. (see **35**).

4. *Fats*

Only the dry weight of the ether extract and the free fatty acid value were estimated. Separate samples were used for fat determinations. The tissue was collected and preserved as previously described, but instead of the alcohol extraction being used as before, the tissue was spread out to dry in a current of air, absolute alcohol being added from time to time to hasten the drying process. The dried tissue was then extracted for 12 hours with absolute ether. Also, the preserving liquid was evaporated, the residue dried *in vacuo*, and taken up with absolute ether. The ether extracts were combined, dried *in vacuo*, and the weight determined. Free fatty acids of this residue were estimated by titration in the usual manner.

5. *Results*

The following statements are made in explanation of the tables of results. Whenever percentages are given, the figures are always in terms of the *total dry* weight of the samples. Also, the numbers of the samples are given in order to facilitate proper comparison

between ethylene treated and untreated tissue; for, as has been stated, a given culture was not divided into the two portions until 72 hours before the end of the cultural period. Considering the unavoidable variations which must enter into different cultures, the treated and untreated pair of samples from one culture should therefore be more comparable than any other combination; for example, the treated sample of one culture and the untreated of another. Samples I and II, afterward IV and V, VI and VII, etc., are directly comparable.

TABLE I

TOTAL ALCOHOL-ETHER SOLUBLE SUBSTANCES IN THE ETHYLENE TREATED AND UNTREATED TISSUE

UNTREATED				TREATED			
No. of sample	Percentage of alcohol-ether soluble	Percentage of alcohol-ether insoluble	Percentage of total solids	No. of sample	Percentage of alcohol-ether soluble	Percentage of alcohol-ether insoluble	Percentage of total solids
I.....	59.79	40.21	8.73	II.....	67.63	32.37	8.54
V.....	59.28	40.72	8.00	IV.....	68.80	31.20	7.82
VII.....	59.04	40.96	7.82	VI.....	67.43	32.57	8.32
XIII.....	59.40	40.60	8.50	XII.....	64.98	35.02	8.60
XV.....	60.00	40.00	7.80	XIV.....	67.60	32.40	8.30
XIX.....	59.60	40.40	8.34	XVIII (*a*)	68.64	31.36	8.90
XXI.....	59.70	40.30	8.53	XVIII (*b*)	68.76	31.34	8.94
XXIII...	59.66	40.37	8.48				
Mean..	59.55	40.45	8.27	Mean..	67.70	32.30	8.48

Table I gives the results of the separation of the samples into alcohol-ether soluble and insoluble portions. Two facts are noteworthy: first, that the soluble substances are more abundant in the ethylene treated than in the untreated tissue, a difference amounting to about 8 per cent; and, secondly, the water content of the two tissues is practically the same, being about 91.5 per cent. To what substances this difference is due should become clearer from the tables to follow.

Table II shows the amount of reducing sugars in the alcohol-ether soluble fraction before and after hydrolysis, and also the reducing power of the insoluble fraction after hydrolysis. These latter data cannot be expressed, even approximately, in percentages,

on account of the probability of a great variety of hydrolyzable polysaccharides present.

The amount of reducing sugars in the soluble fraction is considerably greater in the treated tissue, although the results of sample II are inconsistent. Again, after hydrolysis, the treated tissue still shows more reducing sugar, but the difference is less pronounced, which means that the higher soluble carbohydrates, such as the disaccharides, are really less in this tissue than in the untreated. One seems justified in saying that the ethylene treated tissue has about 11 per cent more of the lower, and about 3 per cent

TABLE II

CARBOHYDRATES

	Alcohol-ether soluble fraction				Alcohol-ether insoluble fraction
	No. of sample	Percentage of reducing sugars before hydrolysis	Percentage of reducing sugars after hydrolysis	Increase by hydrolysis	Mg. cu. reduced for each gm. of material after 2.5 hours' hydrolysis
Untreated tissue	I	.....	.....	.....	153.9
	V	.....	.....	.....	162.2
	VII	10.19	15.92	5.83	143.4
	XIII (*a*)	.....	.....	.....	131.4
	XIII (*b*)	.....	.....	.....	145.2*
	XV	12.30	15.70	3.40	140.1
Ethylene treated tissue.....	II	13.84	15.15	1.31	120.7
	IV	23.18	25.56	2.38	124.6
	VI	23.70	25.30	1.60	109.7
	XIV	20.39	21.51	1.12	118.8

* Hydrolysis 5 hours.

less of the higher soluble sugars than the untreated. The reducing power of the alcohol-ether insoluble fraction after hydrolysis is clearly less in the treated tissue. The polysaccharides, which are likely to be present and which are capable of yielding reducing sugars by this acid hydrolysis, are starch, ligno-celluloses, galactans, pectins, etc. Microchemical tests show that very little starch is present in either tissue. The reducing sugars, therefore, are largely from other polysaccharides. An examination of the drawings of fig. 2 will aid in interpreting the differences found. Around the four leaf traces, mechanical tissue is considerably more abundantly

developed in the untreated epicotyl. This anatomical difference agrees with the findings of KAUFMANN (18) for lupine seedlings treated with ether. From a chemical viewpoint the difference seems sufficient to account for the greater power of reduction of the untreated tissue after hydrolysis, inasmuch as mechanical tissue contains a large proportion of rather easily hydrolyzable carbohydrates. That these polysaccharides are not completely hydrolyzed by the 2.5 hours' hydrolysis is shown by comparing samples XIII (*a*) and XIII (*b*), in which the hydrolyzing time of the latter was doubled.

TABLE III

	No. of sample	Percentage of "crude fiber"	Approximate percentage of cellulose
Untreated tissue	V	15.09	9.2
	VII	16.18	11.4
	XIII (*a*)	15.00	.....
	XIII (*b*)	13.94*	.....
	XV	15.05	.....
Ethylene treated tissue	II	9.22	.....
	IV	11.12	6.22
	VI	11.28	8.3
	XIV	11.66	.....

*Hydrolysis 5 hours.

Table III gives the results of "crude fiber" determinations. "Crude fiber" obviously is a mixture of a large number of substances, such as unhydrolyzed protein, cellulose and other polysaccharides, etc. However, this fiber must be largely cellulose and protein. Less crude fiber was found in the treated tissue. The approximate percentage of cellulose was estimated by subtracting from "crude fiber" the total protein present before hydrolysis, minus the protein rendered soluble by the acid hydrolysis. By this method relative differences, at least, should be shown, and the results indicate that the treated tissue had about 3 per cent less cellulose than the control.

In table IV are shown the total nitrogen and the ammonia and amino nitrogen, before and after hydrolysis, in the alcohol-ether soluble fraction, and the total nitrogen in the alcohol-ether insoluble fraction.

The total nitrogen figures for the former fraction do not show a difference between the treated and untreated tissues. Both the

formol titration and the VAN SLYKE methods show a higher amino nitrogen content in the treated tissue before hydrolysis, but after hydrolysis the difference does not appear. (Determinations after hydrolysis were made only by the VAN SLYKE method.) Since the increase of alpha-NH_2 nitrogen, by hydrolysis, is somewhat more in the untreated tissue, it suggests a less amount of polypeptides in the treated tissue, a situation for the soluble nitrogenous substances corresponding to that of the soluble carbohydrates of this fraction. The ammonia-nitrogen results are very variable and no conclusions can be drawn from them.

TABLE IV

NITROGEN

		Alcohol-ether soluble fraction					Alcohol-ether insoluble fraction	
	No. of sample	Total nitrogen	NH_3 before hydrolysis	Amino-nitrogen before hydrolysis	NH_3 after hydrolysis	Amino-nitrogen after hydrolysis	Total nitrogen	Protein
Untreated tissue	I	5.18	.....	.....	.....	.....	2.19	13.68
	V	5.41	.....	.....	.....	.....	1.91	11.93
	VII	5.07	.....	.....	.....	.....	1.50	9.38
	XIII	.....	.....	1.62*	.....	.....	.....	.....
	XV	.....	.....	1.71*	.....	.....	.....	.....
	XIX	.....	0.32	1.96	.....	.....	.....	.....
	XXI	.....	.....	1.93	.....	2.89	.....	.....
	XXIII	.....	0.76	2.02	1.30	2.78	.....	.....
Ethylene treated tissue	II	5.20	.....	.....	.....	.....	1.82	11.37
	IV	5.62	.....	.....	.....	.....	1.57	9.81
	VI	5.20	.....	.....	.....	.....	0.95	5.93
	XII	.....	.....	2.25*	.....	.....	.....	.....
	XIV	.....	.....	2.34*	.....	.....	.....	.....
	XVIII (*a*)	.....	0.83	2.54	0.93	3.22	.....	.....
	XVIII (*b*)	.....	0.40	2.41	1.25	2.98	.....	.....

*Obtained by the formal-titration method.

The total nitrogen of the alcohol-ether insoluble fraction is less in the treated tissue. By employing the factor 6.25 to the nitrogen figures, it is found that the proteins are about 3 per cent less in the treated tissue. Although the results at first seem somewhat inconsistent, they no longer appear so when treated and untreated samples of the same culture are compared.

Table V gives the percentage of ash of the soluble and insoluble fractions. No marked difference appears between the two tissues.

TABLE V

ASH

	No. of sample	Percentage of ash of the alcohol-ether extract	Percentage of ash of the alcohol-ether insoluble residue
Untreated tissue	I	.....	1.58
	V	.....	1.73
	VII	3.08	1.35
Ethylene treated tissue	II	2.76	1.54
	IV	3.15	1.41
	VI	3.22	1.47

Table VI shows the amount of ether soluble substance and the free fatty acid value. The figures in parentheses were not determined, but calculated on the assumption that the percentage of

TABLE VI

FATS

	No. of sample	Wet wt. of tissue gm.	Dry wt. of tissue gm.	Dry wt. of ether-extract gm.	Percentage of ether-extractives	cc. of N/10 NaOH to neutralize free fatty acid	cc. of N/10 NaOH to neutralize free fatty acid in 1 gm. of ether extract
Untreated tissue	XXVII	119.57	10.043	(0.220)	(2.2)	3.00	(13.63)
	XXIX	171.64	14.417	0.313	2.17	3.86	12.33
Ethylene treated tissue	XXVI	139.45	11.713	(0.140)	(1.2)	1.92	(13.71)
	XXVIII	264.55	22.222	0.271	1.22	3.56	13.13

ether-extractives was the same in these samples as in others. One thing is rather clear from the table, namely that less fat is present in the treated tissue, a fact which agrees with the effects of acetylene in oily seeds as studied by GRAFE and RICHTER (9). The free acid value is of particular interest on account of the claim of IWANOW (13) that the free acid value is predetermined by the degree of saturation of the fatty acids involved in the fat in question. However, the free acid value of the fats in the two tissues was not found

to differ. On the foregoing assumption one may therefore say the nature of the fats in the treated and untreated tissues is the same as regards degree of saturation.

B. ACIDITY

For acidity determinations the epicotyls were collected as described for the chemical analysis. The wet weight of the sample was determined, but instead of being preserved in alcohol, the tissue was directly triturated with water in a mortar. More water was added to bring the mixture up to a definite volume, and finally the free acids present were titrated with N/10 NaOH, using phenolphthalein as indicator. The entire procedure, from the cutting of the seedlings to the end of the titration, required about one hour. The foregoing method is rather unsatisfactory; in addition to the fact that in this way one estimates only the surplus H-ions, other objections may be offered. However, any marked relative difference can be caught by this method.

TABLE VII

ACIDITY

	Wet. wt. of tissue in gm.	cc. of N/10 NaOH to neutralize 1 gm. wet wt. of tissue
Untreated	87.25	0.7241
		0.7482
	25.65	0.8457
		0.8730
Ethylene treated	79.65	0.7706
		0.7313
	29.55	0.8288
		0.8118

The results obtained are found in table VII, expressed in terms of N/10 NaOH required to neutralize 1 gm. wet weight of the tissue. No consistent difference is evident between the treated and control tissues.

C. OSMOTIC PRESSURE AND PERMEABILITY

Osmotic pressure was estimated by two methods, freezing point and plasmolysis. For the former method, the juice was expressed

by means of a hand press giving about 300 kgm. per sq. cm., the tissue having been coarsely cut up with scissors and wrapped in a single layer of art canvas. The freezing point was determined with the BECKMAN apparatus, following the directions given by HAMBURGER (10). Both osmotic pressure and permeability were investigated by the plasmolytic method. Plasmolysis was observed in the cortical cells just underlying the epidermis at the base of the second leaf scale (that is, second from cotyledons). Plasmolytic agents employed were sucrose, glucose, KNO_3, and glycerine. A solution was considered isotonic with the cell sap if it just caused plasmolysis after 30 minutes. The temperature was 20–24° C.

TABLE VIII

OSMOTIC PRESSURE BY FREEZING POINT

	No. of sample	Δ	Pressure in atmospheres	Mean pressure
Untreated	V	0.610	7.33	.....
	VII	0.632	7.60	.....
	XI	0.703	8.46	.....
	.....	.....	.....	7.79
Ethylene treated	IV	0.755	9.08	.....
	VI	0.818	9.84	.....
	VIII	0.827	9.94	.....
	X	0.782	9.41	.....
	XIV	0.821	9.87	.....
	.....	.....	.....	9.63

In table VIII are the results by the freezing point method. It is evident that the juice of the treated tissue has a higher osmotic pressure than that of the control, a difference of about two atmospheres.

Similarly, table IX gives the results by the plasmolytic method. The figures show that the same relative difference of about two atmospheres exists between the treated and untreated tissues, although the pressures themselves are somewhat higher. RICHTER (31) and others have assumed a rise of osmotic pressure in tissues under the influence of anaesthetics. This assumption is based upon the fact that sugars and other osmotically active substances were known to increase. However, no previous measurements

of osmotic pressure under such conditions have apparently been made.

Results with KNO_3 and glycerine indicate probably two things: first, that neither the treated nor the untreated tissues are very

TABLE IX

OSMOTIC PRESSURE AND PERMEABILITY

	Plasmolyzing agent	Concentration gm.-mol.	20–24° C. Osmotic pressure in atmospheres	Difference in gm.-mol. between treated and untreated
Untreated	Sucrose	0.37	9.38–9.52	.....
	Glucose	0.37	.....	.....
	KNO	0.21	.....	.....
	Glycerine	0.43	.....	.....
Ethylene treated	Sucrose	0.46	11.70–11.84	0.09
	Glucose	0.47	.....	0.10
	KNO	0.29	.....	0.08
	Glycerine	0.56	.....	0.13

permeable; and, secondly, that the treated tissue is slightly more permeable than the control.

D. RESPIRATORY CHANGES

For the study of respiration, the cultural methods differed in some respects from those already described. When the epicotyls had become 2–3 cm. long (that is, ready for the usual 72 hours' exposure period), the entire seedlings or the epicotyls only were taken from the pans and placed in test tubes of 20 cc. capacity, graduated for 15 cc. The condition in the test tubes were as follows: They were filled with mercury and inverted over a dish of the same. The mercury in the tubes was displaced to the 15 cc. mark, either with pure air or an ethylene-air mixture containing 0.0002 per cent ethylene. Three entire seedlings or four epicotyls were introduced from below into the various tubes. The experimental periods were 3, 6, 12, 24, 48, and 72 hours. At the close of a period the seedling or epicotyls were withdrawn by means of a hooked wire and the gas present preserved for analysis. The BONNIER and MANGIN apparatus was employed for the gas analysis, following practically the procedure suggested by THODAY (32).

In table X are the results of the analyses. Each set of figures represents the average of a number of analyses; for example, the 12-hour cultures are from 16 analyses upon gas of 3 different cultures. The results plainly show a general depression of respiration by ethylene, both in the CO_2 production and the O_2 absorption. The respiratory ratio gradually increases with the time in both tissues, an increase which probably is due to the lowering of the

TABLE X

RESPIRATORY CHANGES

No. of hours		Untreated tissue			Ethylene treated			
		cc. CO_2	cc. O_2	$\frac{CO_2}{O_2}$	Concentration of gas used, percentage (by vol.)	cc. CO_2	cc. O_2	$\frac{CO_2}{O_2}$
3	Epicotyls only ..	0.2185	0.3664	0.66	0.0002	0.2449	0.3030	0.81
6	" " ..	0.2255	0.3419	0.66	"	0.1703	0.2898	0.61
6	Entire seedling ..	0.4138	0.6069	0.69	"	0.3780	0.5744	0.66
12	" " ..	0.3758	0.5345	0.70	"	0.3197	0.4668	0.69
24	" " ..	0.3587	0.4190	0.84	"	0.3127	0.3873	0.81
24	Epicotyls only ..	0.3145	0.4025	0.78	0.01	0.3380	0.4086	0.84
48	Entire seedling ..	0.2501	0.2897	0.86	0.0002	0.2404	0.2869	0.85
72	" " ..	0.3210	0.2360	1.39	"	0.2610	0.2212	1.04

oxygen pressure. In the 3-hour culture with ethylene the ratio is very large. The result, as it stands, comes from an excessive production of carbon dioxide. This ratio of 0.81 seems extremely high in consideration of the 0.66 ratio of the control, and particularly of the 0.61 ratio of the 6-hour ethylene culture. However, IRVING (**12**) in her study of the effects of chloroform on barley leaves found that "medium" doses cause a large initial outburst of CO_2 quickly followed by a depression.

Conclusion

The results of the present study seem to indicate that the general effect of ethylene on plant metabolism is exactly comparable to the effects of the common anaesthetics, chloroform, ether, etc., as reported by other workers. Also, that the 0.0001 per cent ethylene concentration used is equivalent in its physiological effects to the so-called "strong" concentrations of those anaesthetics,

concentrations which are in reality, for example in the case of chloroform or ether, thousands of times stronger. Probably most of the ether concentrations employed by HEMPEL (11) were many times weaker, physiologically, than the ethylene concentrations of the above-described experiments. Such an assumption would account for the difference between some of her results and those reported in this paper. It seems probable that ethylene, also, would favor condensation processes if used in "weak" or "medium" concentrations.

In the presence of ethylene the simple soluble substances increase at the expense of the higher soluble and insoluble forms; direct reducing sugars against soluble non-reducing sugars and insoluble polysaccharides; amino acids and amids against proteins; and probably fatty acids and glycerine against fats, seeing that the latter were found to diminish. Accordingly, ethylene appears to affect the balance of the general chemical reactions of the plant in favor of the simpler substances. The experimental work offers no evidence as to whether or not this result is accomplished through an acceleration of the hydrolytic as well as through a retardation of the condensation processes, since all the substances present in the tissue examined (epicotyls) had, within a relatively few hours, arrived, in simple translocation forms, from the cotyledons.

The accumulation of soluble substances in the tissue changes the osmotic relations of the cells and may have much to do with the observed swelling of plant organs in the presence of ethylene, for example in the characteristic "horizontal nutation" or "triple response" of the pea epicotyl. Also, the observed retardation of the rate of elongation may partly be accounted for by the fact that the gas interferes with the synthesis of complex substances, that is to say, perhaps with tissue formation.

Summary

1. Ethylene was found to be very effective in producing changes in the general processes of plant metabolism.

2. Chemical analyses showed that ethylene caused the simple soluble substances to increase at the expense of the higher soluble and insoluble forms.

a) The hot alcohol-ether soluble substances (sugars, amino acids, amids, polypeptides, lipoids, etc.) increased by 8–9 per cent, while the insoluble substances (proteins, starch, cellulose, lignocelluloses, etc.) were correspondingly diminished. The water content of the ethylene treated and control tissues was the same.

b) The lower soluble sugars (by direct reduction) were about 11 per cent more and the higher soluble sugars (by reduction after hydrolysis) about 3 per cent less. The reducing power of the alcohol-ether insoluble residue, after hydrolysis, was decidedly less for the ethylene treated tissue; also, the cellulose content was diminished by about 3 per cent.

c) Amino acid plus amids were more, and the polypeptides apparently less in the ethylene treated tissue. The protein content also was about 3 per cent less.

d) Fats were much less abundant in the treated tissue. The free fatty acid value was unchanged.

3. The acidity of the ethylene treated tissue was not found to be changed.

4. Ethylene caused an increase of osmotic pressure, as measured both by the freezing point and plasmolytic methods.

5. The permeability was not sharply affected by ethylene, although it was somewhat increased.

6. Ethylene affected respiration, retarding both the CO_2 production and the O_2 absorption, but the respiratory ratio remained practically the same. An exception to the preceding statement was found in the case of the shortest exposure period (3 hours), in which there occurred, apparently, an excessive production of CO_2, thereby increasing the ratio.

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